CLAIMS

What is claimed is:

1. An isolated polypeptide which associates with a binding partner in a coiled-coil dependent manner wherein said polypeptide comprises a non-natural site sufficient for the addition of a moiety selected from the group consisting of: phosphate (PO_4), ubiquitin, glycosyl, and ADP-ribosyl, and wherein said polypeptide binds to said binding partner in a manner that is dependent upon the addition of said moiety.

2. The isolated polypeptide of claim 1, wherein addition of a said moiety permits association of the corresponding moiety-containing polypeptide with said binding partner.

3. The isolated polypeptide of claim 1, wherein addition of a said moiety prevents association of the corresponding moiety-containing polypeptide with said binding partner.

4. The isolated polypeptide of claim 1, wherein said non-natural site of said isolated polypeptide comprises a contact site which binds to said binding partner, wherein said contact site of said polypeptide is sufficient for the addition of a said moiety.

5. The isolated polypeptide of claim 1, wherein the polypeptide further comprises detection means, said polypeptide comprising said detection means being a reporter molecule.

6. The isolated polypeptide of claim 5, wherein said detection means comprises light emitting detection means.

7. The isolated polypeptide of claim 6, wherein said light emitting detection means emits fluorescent light.

8. The isolated polypeptide of claim 7, wherein said light emitting detection means comprises two different fluorophores.

9 The isolated polypeptide of claim 8, wherein said fluorophores comprise fluorescein and tetramethylrhodamine.

10. The isolated polypeptide of claim 6, wherein said polypeptide comprises a cysteine amino acid through which said light emitting means is attached via a covalent bond.

14. The isolated polypeptide of claim 1, wherein said coiled-coil comprises a said site.

15. The isolated polypeptide of claim 14, wherein said polypeptide associates via said coiled-coil with a second coiled-coil-containing polypeptide to form a dimer.

16. The isolated polypeptide of claim 14, wherein said polypeptide comprises two coiled-coils and therefore may self associate via said two coiled-coils.

17. The isolated polypeptide of claim 14, wherein addition of a said moiety permits association of the corresponding moiety-containing polypeptide with a second coiled-coil-containing polypeptide to form a dimer.

18. The isolated polypeptide of claim 14, wherein addition of a said moiety prevents association of the corresponding moiety-containing polypeptide with a second coiled-coil-containing polypeptide and thus prevents formation of a dimer.

19. A kit for determining the enzyme activity of a selected modifying enzyme in real time comprising an isolated polypeptide comprising a coiled-coil and an engineered site sufficient for the addition of a moiety selected from the group consisting of: phosphate, ubiquitin, glycosyl, and ADP-ribosyl, wherein said polypeptide binds to a binding partner in a manner that is dependent upon the addition of said moiety, and packaging materials therefor.

20. The kit of claim 19, wherein said polypeptide further comprises a site that is adapted to carry a label.

21. The kit of claim 19, wherein the presence of a said moiety on said polypeptide permits association of said moiety-containing polypeptide with said binding partner.

22. The kit of claim 19, wherein the presence of a said moiety on said polypeptide prevents association of said moiety-containing polypeptide with said binding partner.

23. The kit of claim 19, wherein said isolated polypeptide comprises a contact site which binds to said binding partner, and said contact site of said polypeptide comprises a said site sufficient for the addition of a said moiety.

24. The kit of claim 19, wherein the polypeptide further comprises detection means, said polypeptide comprising said detection means being a reporter molecule.

25. The kit of claim 24, wherein said detection means comprises light emitting detection means.

26. The kit of claim 25, wherein said light emitting detection means emits fluorescent light.

27. The kit of claim 26, wherein said light emitting detection means comprises two different fluorophores.

28. The kit of claim 27, wherein said fluorophores comprise fluorescein and tetramethylrhodamine.

29. The kit of claim 25, wherein said polypeptide comprises a cysteine amino acid through which said light emitting means is attached via a covalent bond.

30. The kit of claim 26, wherein said light emitting detection means comprises two different fluorescent proteins.

31. The kit of claim 30 wherein said two different fluorescent proteins comprise green fluorescent protein and red fluorescent protein.

32. The kit of claim 30, wherein said two different fluorescent proteins comprise green fluorescent protein and blue fluorescent protein.

33. The kit of claim 19, wherein said coiled-coil comprises a said site sufficient for the addition of a said moiety.

34. The kit of claim 33, wherein said polypeptide associates via said coiled-coil with a second coiled-coil-containing polypeptide to form a dimer.

35. The kit of claim 33, wherein said polypeptide comprises two coiled-coils and therefore may self-associate via said two coiled-coils.

36. The kit of claim 33, wherein the presence of a said moiety on said polypeptide permits association of the corresponding moiety-containing polypeptide with a second coiled-coil-containing polypeptide to form a dimer.

37. The kit of claim 33, wherein the presence of a said moiety on said polypeptide prevents association of the corresponding moiety-containing polypeptide with a second coiled-coil-containing polypeptide so as to prevent formation of a dimer.

38. A method to monitor the activity of an enzyme comprising the step of monitoring the addition of a moiety selected from the group consisting of: phosphate, ubiquitin, glycosyl, and ADP-ribosyl to a reporter molecule as claimed in claim 5.

39. A method to monitor the activity of an enzyme comprising the step of monitoring the removal of a moiety selected from the group consisting of: phosphate, ubiquitin, glycosyl, and ADP-ribosyl from a reporter molecule as claimed in claim 5.

40. The method of claim 38 or 39, wherein said method further comprises, prior to said step of monitoring, the step of mixing the reporter molecule and its binding partner under conditions which permit binding of said reporter molecule and said binding partner.

41. The method according to claim 38 or 39, wherein said mixing step includes mixing an enzyme that adds to one or both of a said reporter molecule and its binding partner or removes from one or both of a said reporter molecule and its binding

partner a said moiety and measuring the change in energy transfer between said reporter molecule and its binding partner.

42. The method according to claim 41, wherein said measuring is performed by fluorescent resonance energy transfer (FRET).

43. The method of claim 42, wherein said fluorescence emitting means comprise two different fluorophores.

44. The method of claim 43, wherein said fluorophores comprise fluorescein and tetramethylrhodamine.

45. The method of claim 40, wherein said polypeptide comprises a cysteine amino acid through which said fluorescence emitting means is attached via a covalent bond.

46. The method of claim 42, wherein said light emitting means comprises two different fluorescent proteins.

47. The method of claim 46, wherein said two different fluorescent proteins comprise green fluorescent protein and red fluorescent protein.

48. The method of claim 46, wherein said two different fluorescent proteins comprise green fluorescent protein and blue fluorescent protein.

49. The method according to claim 40, wherein said method further comprises exciting said reporter molecules and monitoring fluorescence emission.

50 The method according to claim 41, wherein said enzyme is selected from the group consisting of a kinase, a phosphatase, a UDP-N-Acetylglucosamine-Dolichyl-phosphate-N-acetylsglucosamine phosphotransferase, an O-GlcNAc transferase, a ubiquitin activating enzyme E1, a ubiquitin conjugating enzyme E2, a ubiquitin protein ligase E3, a poly (ADP-ribose) polymerase and an NAD:Arginine ADP ribosyltransferase.

51. The method according to claim 41, wherein said mixing step comprises mixing an agent which modulates the activity of said enzyme.

52. The method according to claim 41, wherein said mixing step comprises mixing an agent which modulates fluorescence emission of said reporter molecule.

53. A kit comprising a fluorochrome-labeled polypeptide of claim 1, an enzyme selected from the group consisting of a kinase, a phosphatase, a UDP-N-Acetylglucosamine-Dolichyl-phosphate-N-acetylsglucosamine phosphotransferase, an O-GlcNAc transferase, a ubiquitin activating enzyme E1, a ubiquitin conjugating enzyme E2, a ubiquitin protein ligase E3, a poly (ADP-ribose) polymerase, and an NAD:Arginine ADP ribosyltransferase, and packaging materials therefor.

54. The kit of claim 53, wherein said kit further comprises a buffer in which said enzyme is active.

55. The kit of claim 53, wherein said kit further comprises a substrate for said enzyme.

56. The kit of claim 55, wherein said substrate is selected from the group consisting of MgATP, cAMP, ubiquitin, nicotinamide adenine dinucleotide (NAD^+), uridine-diphosphate-N-acetylglucosamine-dilichyl-phosphate (UDP-N-acetylglucosamine-dilichyl-phosphate) and UDP-N-acetylglucosamine.

57. The kit of claim 53, wherein said kit further comprises a cofactor for said enzyme.

58. An isolated pair of polypeptides which associate to form a dimer, the pair comprising

a first polypeptide comprising an engineered site sufficient for addition of a moiety selected from the group consisting of: phosphate, ubiquitin, glycosyl and ADP-ribosyl, and detection means; and

a second polypeptide comprising detection means and which is a binding partner of said first polypeptide, whereby the addition/removal of a said moiety to/from said site is detectable via formation of a dimer comprising said first polypeptide with a binding partner.

59. The pair of polypeptides of claim 58 wherein said detection means comprises light emitting detection means.

60. The pair of polypeptides of claim 59, wherein said light emitting detection means emits fluorescent light.

61. The pair of polypeptides of claim 60 wherein said light emitting detection means comprises a first fluorochrome on said first polypeptide and a second fluorochrome different from said first fluorochrome on said second polypeptide, said first and second fluorochromes together being operative to promote fluorescent energy transfer.

62. The pair of polypeptides of claim 61, wherein said fluorochromes comprise fluorescein and tetramethylrhodamine.

63. The pair of polypeptides of claim 59, wherein said first polypeptide comprises a cysteine amino acid through which said light emitting means is attached via a covalent bond.

64. The pair of polypeptides of claim 60, wherein said light emitting detection means comprises two different fluorescent proteins.

65. The pair of polypeptides of claim 64 wherein said two different fluorescent proteins comprise green fluorescent protein and red fluorescent protein.

66. The pair of polypeptides of claim 64, wherein said two different fluorescent proteins comprise green fluorescent protein and blue fluorescent protein.

67. A method of screening for a modulator of enzymatic activity of a kinase, a phosphatase, a UDP-N-Acetylglucosamine-Dolichyl-phosphate-N-acetylsglucosamine phosphotransferase, an O-GlcNAc transferase, a ubiquitin activating enzyme E1, a ubiquitin conjugating enzyme E2, a ubiquitin protein ligase E3, a poly (ADP-ribose) polymerase or an NAD:Arginine ADP ribosyltransferase, the method comprising

a) mixing a candidate modulator, said polypeptide of claim 1 and a binding partner of said polypeptide, wherein each of the polypeptide of claim 1 and said binding

partner comprises detection means for monitoring association/disassociation between said polypeptide and said binding partner, wherein the association or dissociation of said polypeptide and said binding partner is dependent upon the addition to or removal from the polypeptide of claim 1 and/or its binding partner a moiety selected from the group consisting of: phosphate, ubiquitin, glycosyl and ADP-ribosyl, and a sample of material whose enzymatic activity is to be tested; and

b) monitoring association or dissociation of said polypeptide and said binding partner, said association or dissociation being indicative of modulation by said candidate modulator of said enzymatic activity.

68. The method according to claim 67, wherein said detection means comprises light emitting detection means.

69. The method according to claim 68, wherein said light emitting detection means emits fluorescent light.

70. The method according to claim 69, wherein said light emitting detection means comprises two different fluorophores.

71. The method according to claim 70, wherein said fluorophores comprise fluorescein and tetramethylrhodamine.

72. The method according to claim 68, wherein said polypeptide comprises a cysteine amino acid through which said light emitting detection means is attached via a covalent bond.

73. The method according to claim 69, wherein said light emitting detection means comprises two different fluorescent proteins.

74. The method according to claim 73 wherein said two different fluorescent proteins comprise green fluorescent protein and red fluorescent protein.

75. The method according to claim 73, wherein said two different fluorescent proteins comprise green fluorescent protein and blue fluorescent protein.

76. The method according to claim 69, wherein said monitoring comprises measuring the change in energy transfer between said polypeptide and its binding partner.

77. The method according to claim 76, wherein said measuring is performed by fluorescent resonance energy transfer (FRET).

78. A method of screening for a modulator of enzymatic activity of a kinase, a phosphatase, a UDP-N-Acetylglucosamine-Dolichyl-phosphate-N-acetylsglucosamine phosphotransferase, an O-GlcNAc transferase, a ubiquitin activating enzyme E1, a ubiquitin conjugating enzyme E2, a ubiquitin protein ligase E3, a poly (ADP-ribose) polymerase and an NAD:Arginine ADP ribosyltransferase, the method comprising

a) mixing a candidate modulator, said isolated pair of polypeptides of claim 58, wherein said pair of polypeptides comprises detection means for monitoring association/disassociation between said first polypeptide and said second polypeptide, wherein the association or dissociation of said first polypeptide and said second polypeptide is dependent upon the addition to or removal from said first polypeptide and/or said second polypeptide of a moiety selected from the group consisting of: phosphate, ubiquitin, glycosyl and ADP-ribosyl, and a sample of material whose enzymatic activity is to be tested; and

b) monitoring association or dissociation of said first polypeptide and said second polypeptide, said association or dissociation being indicative of modulation by said candidate modulator of said enzymatic activity.

79. The method according to claim 78, wherein said detection means comprises light emitting detection means.

80. The method according to claim 79, wherein said light emitting detection means emits fluorescent light.

81. The method according to claim 80, wherein said light emitting detection means comprises two different fluorophores.

82. The method according to claim 81, wherein said fluorophores comprise fluorescein and tetramethylrhodamine.

83. The method according to claim 79, wherein at least one polypeptide of said pair of polypeptides comprises a cysteine amino acid through which said light emitting detection means is attached via a covalent bond.

84. The method according to claim 80, wherein said light emitting detection means comprises two different fluorescent proteins.

85. The method according to claim 84 wherein said two different fluorescent proteins comprise green fluorescent protein and red fluorescent protein.

86. The method according to claim 84, wherein said two different fluorescent proteins comprise green fluorescent protein and blue fluorescent protein.

87. The method according to claim 80, wherein said monitoring comprises measuring the change in energy transfer between a first polypeptide of said pair of polypeptides and a second polypeptide of said pair of polypeptides.

88. The method according to claim 87, wherein said measuring is performed by fluorescent resonance energy transfer (FRET).

89. A method to monitor the activity of an enzyme comprising the step of monitoring the association or dissociation of a synthetic polypeptide and its binding partner wherein said synthetic polypeptide comprises an amino acid which is covalently linked to a moiety selected the group consisting of: phosphate, ubiquitin, glycosyl and ADP-ribosyl wherein said synthetic polypeptide and its binding partner associates or dissociates in a manner that is dependent upon the removal from said synthetic polypeptide of said moiety.

90. The method according to claim 89, wherein said synthetic polypeptide and said binding partner thereof each comprises light emitting detection means.

91. The method according to any of claims 38, 39, 67, 78 and 89, wherein said method comprises real-time observation of association of a said isolated polypeptide and its binding partner or of a said isolated pair of polypeptides.